

# THE IMPORANCE OF MITIGATING ANALYTE INTERACTIONS WITH METAL SURFACES IN HPLC SEPARATIONS OF SMALL MOLECULES

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## INTRODUCTION

Interactions of certain analytes with metal surfaces in UPLC™ Instruments and Columns are known to cause a range of deleterious effects including peak tailing, low peak areas [1] and the formation of new peaks due to on-column reactions [2]. To mitigate these effects, we have developed a novel surface modification technology in which a hybrid organic/inorganic composition based on an ethylene-bridged siloxane chemistry is formed on the metal components in UPLC Instruments and Columns [3]. This technology has been shown to give reduced tailing, higher peak areas and lower injection-to-injection variability for metal-sensitive analytes such as nucleotides [3], tricarboxylic acid cycle metabolites [4], oligonucleotides [5,6], acidic peptides [7] and small molecule pharmaceuticals [8,9]. Here we demonstrate the benefits of this technology for UPLC Separations of several small molecule pharmaceuticals.

## METHODS

Standard UPLC Instruments and Columns were compared to versions employing hybrid surface technology (HST), designated as ACQUITY™ Premier Systems and Columns.

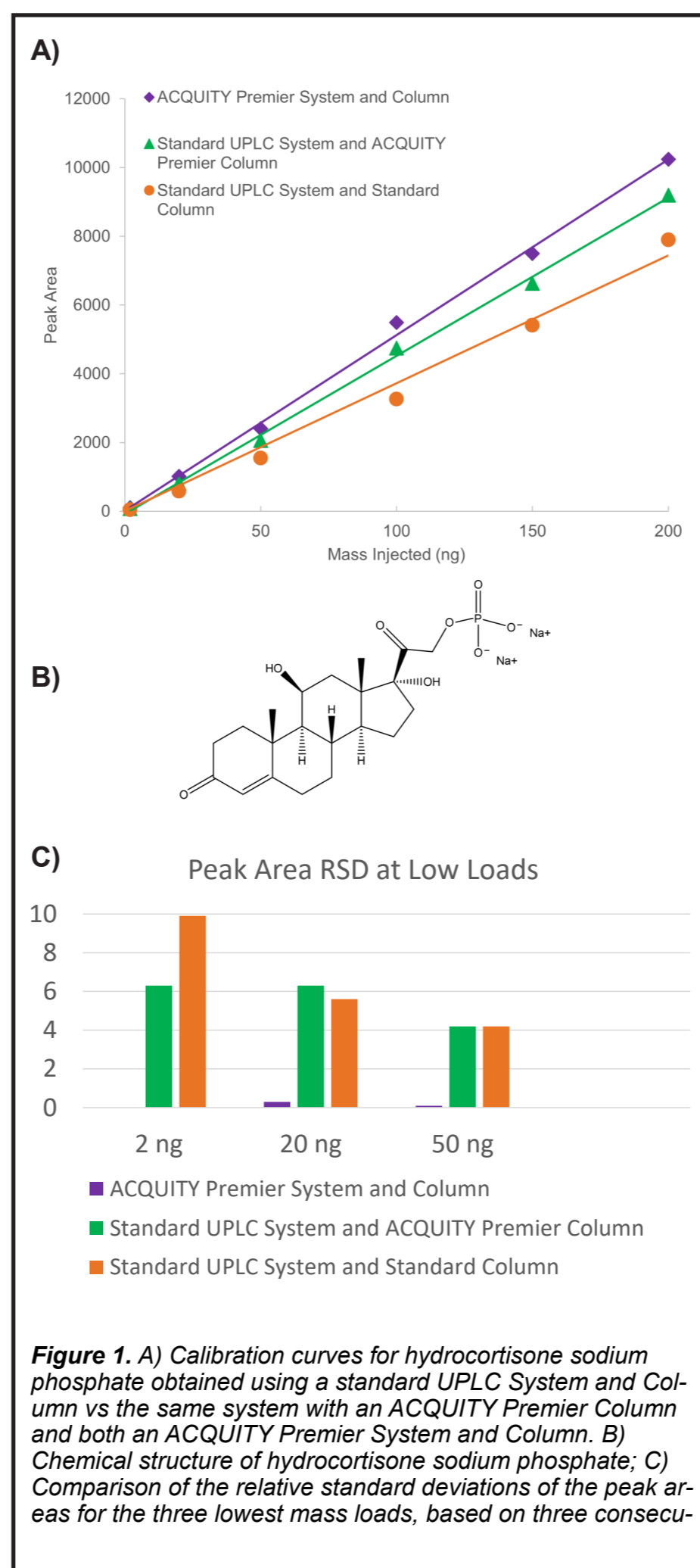
1. Separations of hydrocortisone sodium phosphate were carried out using both an ACQUITY Premier System and a standard UPLC System with ACQUITY UPLC BEH™ C<sub>18</sub> 1.7 μm 2.1 x 50 mm Columns and an acetonitrile gradient using a mobile phase containing 10 mM ammonium formate (pH 3.0). UV detection was employed to determine peak area as a function of mass injected over the 2 – 200 ng range. Triplicate injections were made for each mass to determine the variability in the peak areas.
2. Separations of deferoxamine mesylate were carried out using an ACQUITY Premier System with ACQUITY UPLC HSS T3 1.8 μm 2.1 x 50 mm Columns and an acetonitrile gradient using an aqueous mobile phase containing 10 mM ammonium formate (pH 3.0). Electrospray ionization mass spectrometry detection was used to determine peak area as a function of mass injected over the 2 – 60 ng range. Ten consecutive injections were made at the 10 ng mass load to determine the variability in the peak areas. For the other mass loads, triplicate injections were made.
3. Separations of clozapine were carried out using a standard UPLC System with ACQUITY UPLC BEH C<sub>18</sub> 1.7 μm 2.1 x 50 mm Columns and an acetonitrile gradient using an aqueous mobile phase containing 10 mM ammonium hydroxide. UV detection was used to determine peak areas of clozapine and its N-oxide over a series of injections.

### References

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## RESULTS AND DISCUSSION

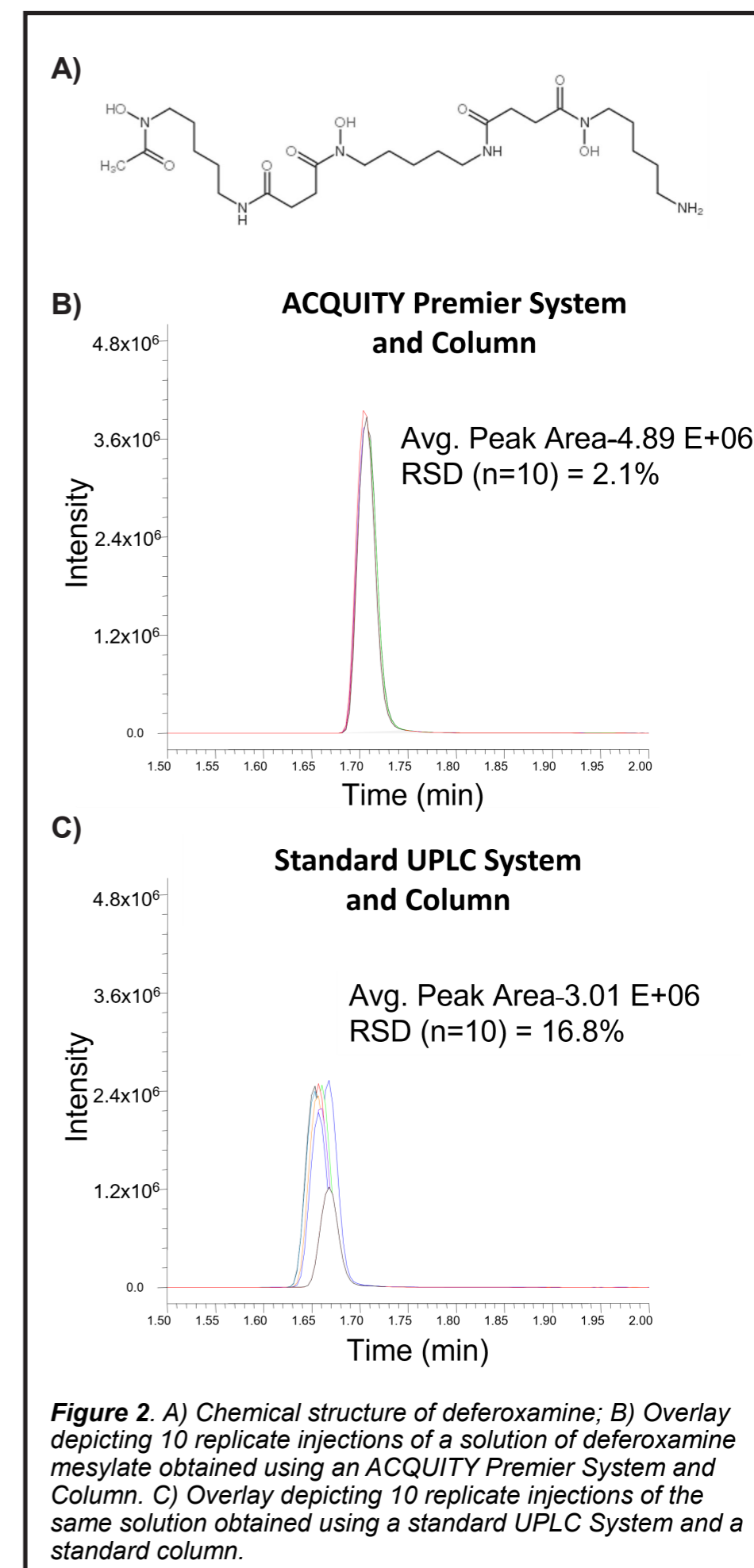
The results of the comparison of a standard UPLC System and Column vs hybrid surface technology (HST) versions (ACQUITY Premier System and Column) for hydrocortisone sodium phosphate showed that the HST versions produced narrower, more symmetric peaks with higher and more reproducible peak areas (shown in Figure 1). Relative to the standard system and column, the slopes of plots of peak area vs mass injected were greater for the HST System and Column and the peak area relative standard deviations (RSD) were greatly reduced for the lowest mass loadings (2, 20 and 50 ng). The results of a similar standard vs HST System and Column study for deferoxamine mesylate also show higher peak areas and reduced peak area variability when using an HST System and Column (shown in Figure 2). The peak area RSD for ten consecutive injections using an HST System and Column at a mass load of 10 ng was 2.1%, vs 16.8% when using a standard system and column. The results for clozapine (Figure 3) showed that the N-oxide formed by on-column oxidation was observed with a 2.05% relative peak area after 13 injections when using a standard column. In contrast, the N-oxide was observed with a 0.06% relative peak area after the same number of injections on an HST Column.



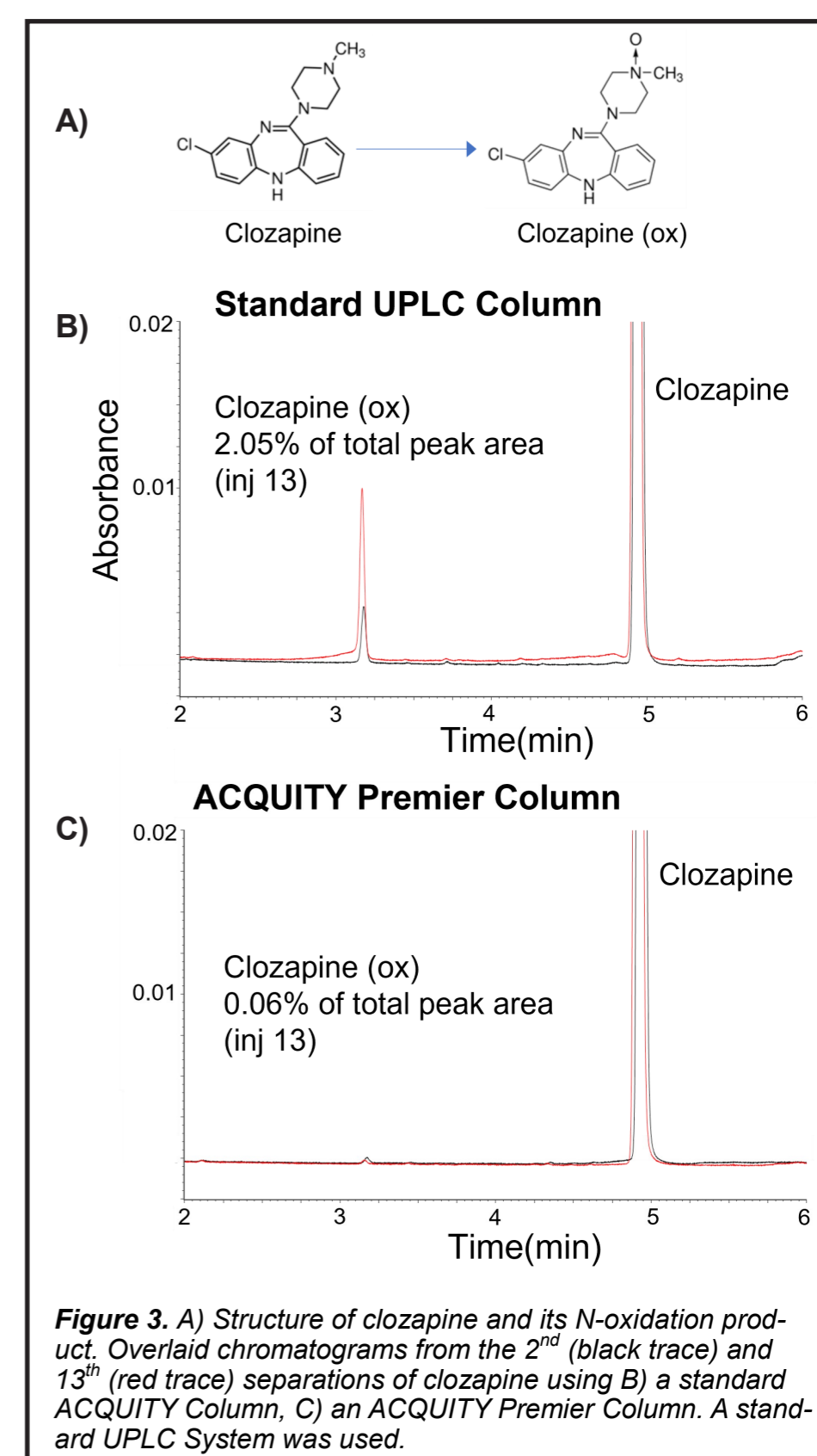
**Figure 1.** A) Calibration curves for hydrocortisone sodium phosphate obtained using a standard UPLC System and Column vs the same system with an ACQUITY Premier Column and both an ACQUITY Premier System and Column. B) Chemical structure of hydrocortisone sodium phosphate; C) Comparison of the relative standard deviations of the peak areas for the three lowest mass loads, based on three consecutive injections.

## CONCLUSION

These results demonstrate that interactions with metal surfaces in UPLC Instruments and Columns can affect the ability to obtain accurate and reproducible results for some small molecule pharmaceuticals. The effects caused by these interactions include peak tailing, reduced peak areas and greater peak area variability. In addition, under some conditions pharmaceutical analytes may undergo on-column reactions catalyzed by metal surfaces. This results in the observation of peaks from compounds that are not present in the sample. We have demonstrated that the use of hybrid surface technology applied to the metal surfaces in UPLC Systems and Columns mitigates these effects. This enables more accurate and reproducible results for analytes that are susceptible to interactions with metal surfaces.



**Figure 2.** A) Chemical structure of deferoxamine; B) Overlay depicting 10 replicate injections of a solution of deferoxamine mesylate obtained using an ACQUITY Premier System and Column. C) Overlay depicting 10 replicate injections of the same solution obtained using a standard UPLC System and a standard column.



**Figure 3.** A) Structure of clozapine and its N-oxidation product. Overlaid chromatograms from the 2<sup>nd</sup> (black trace) and 13<sup>th</sup> (red trace) separations of clozapine using B) a standard ACQUITY Column, C) an ACQUITY Premier Column. A standard UPLC System was used.

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